AN OPEN LABEL IN-VITRO, IN-VIVO STUDY TO EVALUATE THE ANTI-INFLAMMATORY ACTION OF GUGGULUTHIKTAM KASHAYA AND SIMHANADA GUGGULU FOLLOWED BY CLINICAL STUDY IN RHEUMATOID ARTHRITIS

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By

Dr SREELEKSHMI.G.U.

Ph.D

(18AYU6AYU1107)

Supervisor

Dr RATNAPRAVA MISHRA

Professor, Head

Dept. of Kayacikitsa

MANDSAUR UNIVERSITY

Approved by UGC, Rewas Dewda Road, SH - 31, Mandsaur, Madhya Pradesh- 458001

Ph. No. +919752122999, +919425924111

Website: www.mandsauruniversity.edu.in/

Email : info@mandsauruniversity.edu.in, phd@mandsauruniversity.edu.in

AN OPEN LABEL IN-VITRO, IN-VIVO STUDY TO EVALUATE THE ANTI-INFLAMMATORY ACTION OF GUGGULUTHIKTAM KASHAYA AND SIMHANADA GUGGULU FOLLOWED BY CLINICAL STUDY IN RHEUMATOID ARTHRITIS

ABSTRACT :

Rheumatoid Arthritis is a chronic disease, which affects the quality of life of people. It starts as an inflammatory condition of the joints, progresses as synovitis, proliferation of synoviocytes, infiltration of the polymorphs, leukocytes etc. and leads to bone erosion and ends in deformities such as swan neck, boutonniere, z – thumb deformity etc. The clinical manifestations includes tenosynovitis, morning stiffness greater than 30 minutes, loss of Appetite, loss of Weight, mild fever etc. Smaller as well as large joints will get affected. On hematological investigation, Erythrocyte sedimentation rate, Rheumatoid Factor etc. will be raised. In Ayurveda similar conditions are explained by Acharyas like Amavata, Vataraktha etc. and many effective medicines are advocated for their cure. Gugguluthikthakam Kashayam and Simhanada Guggulu are two such renowned formulations. Although many NSAIDS and DMARDS could manage the symptoms of Rheumatoid Arthritis, side effects can occur by its prolonged use. Cost effective and clinically effective drugs from Ayurveda is essential for chronic disease like RA where prolonged anti-inflammatory management is necessary. This study is a humble effort to assess the efficacy of Gugguluthiktam kashaya and Simhanada Guggulu Gulika in Rheumatoid Arthritis through in-vitro, in-vivo studies followed by the clinical phase.

Keywords : Gugguluthikthakam Kashayam, Simhanada Guggulu, Rheumatoid Arthritis

OUTLINE OF THE PROPOSED RESEARCH WORK

I. Title of the Research Work:

An Open Label In-Vitro, In-Vivo Study To Evaluate The Anti-Inflammatory Action Of Gugguluthiktam Kashaya And Simhanada Guggulu Followed By Clinical Study In Rheumatoid Arthritis

II. Importance/ Rationale of Proposed Investigation

Rheumatoid Arthritis (RA) is a chronic, systemic inflammatory illness that primarily attacks peripheral joints and the surrounding muscles, tendons, ligaments, and blood vessels. Partial remissions and unpredictable exacerbations mark the course of this potentially crippling disease. RA can occur at any age, but it begins most often between ages 25 and 55. The female-to-male ratio is 3:1.[1] The mechanisms of RA include abnormal immune activation (in a genetically susceptible person) leading to inflammation, complement activation and cell proliferation in joints and tendon sheaths. Infection (viral or bacterial), hormone action, or lifestyle factors – smoking etc. can also be the risk factors. The body produces immunoglobulin (Ig)M antibody against the body's own IgG (also called rheumatoid factor [RF]). RF aggregates into complexes, generates inflammation, causing eventual cartilage damage and triggering other autoimmune responses. If not arrested, the inflammatory process in the joints occurs in four stages : congestion and edema of the synovial membrane and joint capsule cause synovitis. Infiltration by lymphocytes, macrophages, and neutrophils continues the local inflammatory response. These cells, as well as fibroblast – like synovial cells, produce enzymes that help to degrade bone and cartilage. Pannus – thickened layers of granulation tissue – covers and invades cartilage and eventually destroys the joint capsule and bone. Fibrous ankylosis – fibrous invasion of the pannus and scar formation – occludes the joint space. Bone atrophy and misalignment cause noticeable deformities and disrupt the articulation of opposing bones, causing muscle atrophy and imbalance and, possibly, partial dislocations (subluxations). Fibrous tissue calcifies, leading to bony ankylosis and total immobility.

Initial signs and symptoms are nonspecific (most likely related to the initial inflammatory reactions) precede inflammation of the synovium, including fever, weight loss, malaise, fatigue, and lymphadenopathy. As the disease progresses, specific localized, bilateral, and symmetric articular symptoms, frequently within the fingers at the proximal interphalangeal, metacarpophalangeal, and metatarsophalangeal joints, possibly extending to the wrists, knees, elbows, and ankles from the inflammation of the synovium. Stiffening of affected joints after inactivity, especially on arising in the morning. Joint pain and tenderness, at first only with movement but eventually even at rest. Flexion deformities or hyperextension of metacarpophalangeal joints, subluxation of the wrist, and stretching of tendons pulling the fingers to the ulnar side (ulnar drift), or characteristic "swan's neck" appearance or "boutonniere" deformity. Extra-articular findings include Gradual appearance of rheumatoid nodules – subcutaneous, round or oval, nontender masses (20% of RF – positive patients), usually on elbows, hands, or Achille's tendon. Vasculitis possibly leading to skin lesions, leg ulcers, and multiple systemic complications. Pericarditis, pulmonary nodules or fibrosis, pleuritis, or inflammation of the sclera and overlying tissues of the eye. Peripheral neuropathy with numbness or tingling in the feet or weakness and loss of sensation in the fingers.

Although nonsteroid anti-inflammatory drugs and disease modifying drugs could manage the symptoms of Rheumatoid Arthritis, side effects can occur by its prolonged use. Cost effective and clinically effective drugs from Ayurveda is essential for chronic disease like RA where prolonged anti-inflammatory management is necessary.

The inflammatory process and disease symptoms are similar to the vata vyadhis (diseases) mentioned in Ayurveda like Amavata, Vataraktha etc. In Amavata, angamardha (body ache), Aruchi (Anorexia), trishna (thirst), aalasyam (langour), gauravam (heaviness), jwara (fever), apaka (indigestion of the ingested food), shoonata anganam (generalized oedema) are told as the features. The complications develops and causes painful swelling in the joints of hands, feet, head, ankle, sacral joint, knee, thigh etc. Hrdgraha, constipation, stiffness of the body parts etc. also develops. In Vataraktha, sopha, ruja etc. and sandhi-asthi vaikalyam are told.

In Ayurveda medicine, several formulations are indicated as sothaharam and for sandhi-asthi-majja gata conditions. Gugguluthiktham kashaya[2] (mentioned in Vatavyadhi prakarana of Ashtanga Hridaya) and Simhanada Guggulu[3] (mentioned in Amavata Prakarana) are such medications which can be used effectively for this condition, which can be proven with the aid of modern diagnostic and assessment criterias. Gugguluthiktam kashayam is indicated for sopha, sandhi-asthi-majja gata vyadhi and panduroga which is having relevance with the disease Rheumatoid Arthritis. This study is a humble effort to assess the efficacy of Gugguluthiktam kashaya and Simhanada Guggulu Gulika in Rheumatoid Arthritis through in-vitro, in-vivo studies followed by the clinical phase.

III. Scope of Proposed Investigation

1) Validation of effective anti-inflammatory drugs mentioned in Ayurveda for the treatment of Rheumatoid Arthritis.
2) The outcome expected can be change in the objective criterias - RA Factor, ESR, CRP, DAS-28 Score (Disease Activity Score 28)[4] and VAS Score.
3) The anti-inflammatory action of Gugguluthiktam kashaya and Simhanada guggulu in in-vitro, in-vivo and clinical level can be evaluated

IV Location:

a) Organization/Department where the work is to be done : Mandsaur institute of Ayurveda and Research Centre, and other eligible centres for in-vitro, animal study and drug formulation etc.

b) Geographical Area of Investigation, if any: Mandsaur institute of Ayurveda and Research Centre, and other eligible centres for in-vitro, animal study and drug formulation etc.

V Review of Work done on the Subject:

In-vivo and in-vitro studies on classical drugs like Gugguluthiktam kashayam and Simhanada Guggulu **with its clinical efficacy** on Rheumatoid Arthritis is not done till the date.

- **Sources of Information:** Research Databases(Pubmed, Dhara, Ayush portal etc.), Classical Text Books of Ayurveda and Modern Text books
- **Tools and Techniques of Research:** Keywords, Boolian operators

VI Research gaps identified in the proposed field of investigation:

- Preliminary datas – in-vivo and in-vitro studies on classical drugs like Gugguluthiktam kashayam and Simhanada Guggulu **with its clinical efficacy** on Rheumatoid Arthritis is not done till the date.

1. **Aswathy et al.** was a validated study about the poly herbal kashayams like Gugguluthiktam kashayam etc. but it was not continued up to the clinical phase and hence further documentation with the assessment parameters for Rheumatoid Arthritis was not done.
2. **Sasane P et.al.** studied the clinical evaluation of Alambushadi Ghana Vati and Vaitarana Basti in the Amavata management, with special reference to Rheumatoid Arthritis. Statistically, highly significant (HS) improvement was found in erythrocyte sedimentation rate , and HS results were found in symptoms of Amavata when the Vaitarana Basti was used along side Alambushadi *Ghana Vati*. DAS28 Score – methodological limitations, in vitro-in vivo studies were not done
3. **Gupta SK et.al.** studied a single case about the management of Amavata (rheumatoid arthritis) with diet and Virechanakarma and the result suggested that *Amavata*/ RA is managable with appropriate diet regimen, *Virechanakarma* But there is *Methodological limitations for the study.*
4. **Pandey SA.et.al.** studied for the clinical efficacy of two drugs - Shiva Guggulu and Simhanada Guggulu in Amavata (Rheumatoid Arthritis) in which 24 patients of Amavata were randomly grouped into two. In group A, Shiva Guggulu 6 g/day in divided doses and in B , Simhanada Guggulu 6 g/day in divided doses and were given for 8 weeks. On study of the results, it had been found that Simhanada Guggulu provided better results as compared to *Shiva Guggulu* in the management of *Amavata*. In both the groups, none of the patients were found to be completely cured because of the short duration course of therapy as well as chronic nature of the disease. Also the drugs were not validated.

VII Objective of the proposed study

1) To evaluate the anti-inflammatory activity by using suitable in-vitro (Protein denaturation assay/ Trypsin inhibitory activity/ Membrane stabilizing Activity) and in-vivo (FCA – Freund's Complete Adjuvant induced Arthritis/ Pristane Induced Arthritis) models. Determination of various biochemical parameters supporting the anti-inflammatory activity like TNF α and Interleukin.

2) To evaluate the anti-inflammatory action of gugguluthiktam kashaya and simhanada guggulu by clinical study in rheumatoid arthritis through the changes in the parameters - RA Factor, ESR, CRP, DAS-28 Score (Disease Activity Score 28), and Vas Score.

VIII. Research methodology:

- **Hypotheses to be tested:**

  "Whether Gugguluthiktam kashaya and Simhanada guggulu having anti-inflammatory action and are effective in Rheumatoid Arthritis" ?

  Null Hypothesis (H_0) : Gugguluthiktam kashaya and Simhanada guggulu, not having anti-inflammatory action and aren't effective in Rheumatoid Arthritis.

  Alternate Hypothesis (H_1) : Gugguluthiktam kashaya and Simhanada guggulu having anti-inflammatory action and are effective in Rheumatoid Arthritis.

- **Inclusion and exclusion criteria of patients**

  - ✓ Inclusion Criteria : Eligible subjects either male or female with diagnosed RA according to the revised 2010 American College of Rheumatology (ACR) criteria and Disease Activity Score (DAS).
  - ✓ Exclusion criteria : Deformities and malformations of Musculoskeletal system, Pregnancy and lactation and RA Secondary to any disease will be excluded.

- **Plan of Work :**

  STUDY DESIGN: Open Label In-Vitro, In-Vivo, Clinical Study

  STEP 1 : The medicinal formulations will be prepared from a GMP certified company.

  STEP 2 : The anti-inflammatory studies, in-vitro and in-vivo studies will be conducted in a pharmacy with all eligible facilities.

  STEP 3 : The clinical study of patients will be conducted under the guidance of Dr.Ratnaprava Mishra., M.D., PhD.

  STEP 4 : Men and women aged 20 to 60 years are selected - according to ACR DIAGNOSTIC CRITERIA – 2010[5]

STEP 5 : Clinical intervention

Shamana oushadhi from the 1st day onwards	Gugguluthiktam kashayam, Simhanada guggulu gulika Dose & Duration will be fixed as per the classical guidelines[6] and according to the in vitro – in vivo study

STEP 6 : OUTCOME MEASURES:

Subjective assessment	Pain (Visual Analogue Scale), Early Morning Stiffness (Minutes)[7]
Objective assessment	ESR, CRP, RF Factor, Das 28(Disease Activity Score 28)

STEP 7 : Statistical approach: Appropriate statistical measurements will be used for the analysis

IX. Expected outcome of research :

The outcome expected is a change in the criterias - ESR, CRP, RF Factor, Das 28 (Disease Activity Score 28), Vas Score.

X. Tentative Chapterization : Introduction, rationale, review of literature, design and methodology

XI. Bibliography

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